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It is characteristic of Science and Progress that they continually open new fields to our vision.—PASTEUR.

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THE SO-CALLED REDUCED OXYGEN TENSION FOR GROWING THE MENINGOCOCCUS

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The following series of experiments was planned to determine the rôle that carbon dioxide plays in the cultivation of the meningococcus. Although they have the limitation of dealing only with two strains, they emphasize the importance of the use of this gas in culturing the meningococcus within certain reactions of the media. The possibility was considered that definite knowledge as to the exact effect of this gas on such cultures might lead to some application of the principle involved to the treatment of meningococcus infections.

Wherry and Oliver (1916) found that thousands of colonies of the gonococcus could be produced by growth at partial oxygen tension when control aerobic tubes remained sterile or showed only a few colonies. In accordance with this Cohen and Markle (1916) made aerobic, partial tension and anaerobic cultures from a case of cerebrospinal meningitis on a medium composed of equal parts of the basic sodium phosphate agar described by Martin (1911), and sterile pleuritic fluid. The pyrogallic acid method was used for the anaerobic cultures, and for the partial tension the culture was connected to a freshly inoculated agar slant of *B. subtilis* by means of a rubber tube. Two colonies appeared on the aerobic tubes, whereas hundreds appeared on the partial tension tubes. The anaerobic cultures remained sterile. In a later article Cohen (1918) again reports favorably on this method and draws the conclusion that the meningococcus is a micro-aerophil, and that the growth ordinarily obtained by aerobic methods consists only of the small minority of meningococci that are resistant to full oxygen tension. Accordingly

Cohen and Fleming (1918) have worked out the optimum amount of air to be replaced by carbon dioxide to culture the meningococcus. Their method has been in use for about six months in the laboratory of the Base Hospital at Camp Jackson. It consists in placing 5 grams of sodium carbonate in a beaker in a 10 liter museum jar containing the cultures either in tubes or on plates. A solution of 5 cc. of sulphuric acid in 35 cc. of water is poured into the beaker and as soon as the violent reaction begins to subside, the cover is clamped down. A tin pail with a close fitting cover has subsequently been used with equally good results. The carbon dioxide thus generated will replace about 10 per cent of the air.

St. John (1919), in a recent paper, concludes that the only advantage in growing the meningococcus in connection with *B. subtilis* and in a partial tension of carbon dioxide comes from the increased moisture in the atmosphere and on the surface of the media under these conditions. He partly replaced the air in Novy jars by means of hydrogen so that the oxygen content varied between 15.6 per cent and 1.2 per cent. He also reduced the oxygen tension by means of *B. subtilis* to amounts varying between 15.3 per cent and 9.5 per cent of atmospheric tension, and reduced the air pressure by means of a vacuum in various degrees. He compared growths obtained under these conditions with that on plates in an atmosphere of air, as well as on plates in a sealed chamber containing moisture. His conclusions are that the moist chamber gives as good or better growth than the other methods. He states that growth is inhibited by carbon dioxide when more than 50 per cent of the air is replaced, but does not say anything as to the effect of the amount recommended by Cohen and Fleming.

It is no doubt true that a moist atmosphere is very beneficial for the growth of the meningococcus, as it probably is in the case of most organisms. But the results of the experiments reported below are in accord with the findings of Cohen and Fleming that the replacement of air by carbon dioxide has a very stimulating effect on the growth of the meningococcus under certain conditions. It did not seem, however, that the

effect could be due to reduced oxygen tension as they claim. The atmosphere contains nearly 21 per cent of oxygen. Replacing 10 per cent of the air with carbon dioxide reduces this figure to between 18 and 19 per cent. The resulting change in pressure is scarcely appreciable as it is within the variations of barometric pressure. But 10 per cent of carbon dioxide in the air is many times the normal figure and carbon dioxide is by no means an inert gas.

In seeking for explanations two other possibilities seemed worthy of consideration in accounting for the effect of carbon dioxide on the growth of meningococcus. It could not be disregarded that the gas might be one of the nutrients necessary for this organism. Such a case would not be without parallel as Nathanson (Loeb, 1917), as well as Winogradsky, have shown. Wherry and Ervin (1918) state that if carbon dioxide is removed from the atmosphere of a culture of the tubercle bacillus, growth is inhibited. Chapin (1918) claims that carbon dioxide has a very beneficial effect on the growth of the gonococcus other than its influence on the reaction of the media in as much as it stimulates growth even in acid reaction. But he does not give the hydrogen ion concentration of the acid reaction used by him. On the other hand the effect of this gas on the reaction of the media seemed the most plausible explanation. The importance of this phase of nutrient media is just beginning to be realized. Cohen and Clark (1918) have recently shown that even in the case of the more common bacteria, *B. bulgaricus*, *B. coli*, *B. aerogenes*, *B. proteus*, *B. dysenteriae*, (Flexner and Shiga), the position of the optimal zone varies enough to prohibit the use of a common reaction.

Let us consider what happens in a slightly alkaline media under a given pressure of carbon dioxide. The gas will be absorbed until the free alkali is converted into sodium bicarbonate. After this reaction has gone to completion the gas will still be absorbed to form carbonic acid, the final concentration depending upon the pressure. A buffer system thus results like that which is so effective in maintaining the constancy of the reaction of the blood stream and other biological fluids, where

the carbon dioxide tension is maintained by the respiratory system. Considerable variation in the pressure does not materially affect the reaction, because appreciable variation in the ratio, H_2CO_3: $NaHCO_3$, produces but little change in the hydrogen ion concentration. The result then, of an atmosphere containing a certain tension of carbon dioxide, on media of varying degrees of alkalinity is first to reduce all to the same reaction, and then maintain it by a very delicately adjusted buffer system; for as soon as acid substances are produced by the growing organism, an equivalent amount of carbon dioxide is evolved and the reaction remains unchanged.

In the following experiments 2.5 per cent slant agar tubes made with meat infusion and containing 1 per cent Bacto-peptone and 4 per cent defibrinated human blood were used. The hydrogen ion concentration was determined by comparison with standard solutions of known hydrogen ion concentration. For the titration a "comparator" block was used to hold the tubes as represented in the following diagram.

	Media	*Water*	*Media*
Second row	4	5	6
	Standard	*Media*	*Standard*
First row	1	2	3

Into the tubes numbered 2, 4, and 6 was put 5 cc. of the media and this was diluted to 15 cc. Into the tubes 1 and 3 was put 5 cc. of the standard solution, also diluted to 15 cc. Tube 5 contained distilled water. At 1 was placed the standard solution with which the media were to be compared. For 3 one tube was prepared of the standard above and one of the standard below that in 1. These two were interchanged as a guide to get a closer comparison between the media and the standard in 1. Into tubes 1, 2 and 3 were put equal amounts of the particular indicator best adapted to the range desired as recommended by Clark and Lubs (1917). Other variable contents of the media will be noted in the different experiments as they are described. For incubation all tubes were placed in 10 liter museum jars which had moisture in the bottom as well as a tumbler containing water. Vaseline was used to make the

covers fit closely on these jars but no attempt was made to clamp them down to maintain a pressure. To produce an atmosphere containing approximately 10 per cent carbon dioxide, 5 grams of sodium carbonate were placed in a tumbler in the jar and a solution of 5 cc. of sulphuric acid in 35 cc. of water poured over it, the cover being placed on the jar as soon as the reaction began to subside. This will be designated the CO_2-jar. By air-jar is meant a similar jar with similar moisture conditions but without the carbon dioxide.

A strain of meningococcus was first used which was obtained from the blood culture of a case of meningococcus septicemia. When this culture was twenty-four hours old it was transplanted to brain media (ground beef brain with enough meat infusion broth added to cover the brain after being autoclaved) and incubated without carbon dioxide. By propagating the organism in this medium throughout the experiments, a uniform inoculating material was used and the organism did not become accustomed to an atmosphere of carbon dioxide as the experiments progressed. To inoculate the tubes, 0.2 cc. of the supernatant liquid of a brain media culture was emulsified with 5 cc. of broth and into each tube was introduced a uniform drop of this emulsion. This drop, with the water of condensation in the butt of the agar slant, gave an abundance of liquid to flood the entire slant by properly tipping it, which resulted in a uniform seeding and gave a uniformly moist medium. A system of + signs is used to designate the degree or heaviness of growth. One + means the lightest growth obtained in that particular experiment in which it occurs. Five + is the heaviest growth recorded and in that case the individual colonies were 0.4 cm. in diameter. Two + is a satisfactory growth whereas three + and four + both represent a heavy growth.

Experiment I. February 1, 1919. When the first brain media culture was twenty-four hours old it was used to inoculate six blood agar slants, containing 1 per cent glucose, of each of the given reactions. Three of the tubes of each reaction were incubated in the air-jar and the other three in the CO_2-jar. The results after eighteen hours are given in table 1.

TABLE 1

	REACTION OF MEDIA					
	pH = 7.6	pH = 7.8	pH = 8.0	pH = 8.2	pH = 8.4	pH = 8.6
Growth in air-jar	0	0	0	0	0	0
Growth in Co_2-jar	+++	+++	+++	++++	+++	+

This experiment was repeated the next day when the inoculating material was forty-eight hours old with the same results.

Experiment II. February 3, 1919. This experiment is a repetition of experiment I with the exceptions that the inoculating culture was seventy-two hours old, and that all the slants, six for each reaction, were set up in the CO_2-jar with 10 per cent carbon dioxide a day previous to inoculation. After inoculation three tubes of each reaction were incubated in the CO_2-jar and three in the air-jar. The results after twenty-hours are tabulated in table 2.

TABLE 2

	REACTION OF MEDIA					
	pH = 7.6	pH = 7.8	pH = 8.0	pH = 8.2	pH = 8.4	pH = 8.6
Growth in air-jar	++	+*	0	0	0	0
Growth in CO_2-jar	+++	+++	+++	+++	+++	++

*Only one of the three tubes showed any growth.

In these two experiments the media were the same. It will be noticed that the only conditions under which growth was obtained in an atmosphere of air was for the reaction pH = 7.6 and 7.8 when these tubes had first been kept in a CO_2-jar for a day previous to inoculation. But the growth with these reactions in the air-jar was distinctly inferior to that in similar tubes incubated in the CO_2-jar. Evidently enough carbon dioxide was absorbed the day previous to inoculation to induce some growth by partially adjusting the reaction. This experiment was repeated twice later and in both cases similar results were obtained. From the laws of the solubility of gases it follows that as soon as the tubes are removed from the CO_2-jar they begin to lose the gas absorbed by the media in excess of that which combines with the free alkali, and thus the buffer effect of the carbon dioxide is in part lost. This explains why a less

favorable growth resulted in air even though the media had been in the CO_2-jar on the day previous to inoculation.

Experiment III. February 7, 1919. Six blood agar slants with a reaction of pH = 8.2 and containing 1 per cent glucose, and six of the same lot of media but lacking the glucose were inoculated with a twenty-four hour brain media culture. Three of the glucose and three of the glucose-free tubes were incubated in the air-jar and three in the CO_2-jar. After twenty hours incubation all the tubes in the CO_2-jar had a heavy growth of meningococcus, no difference being noticeable between the glucose and the glucose-free media. In the air-jar no growth was obtained and none developed upon twenty-four hours subsequent incubation in the CO_2-jar. Evidently the carbohydrate cannot supply the carbon dioxide or act as a substitute for it under the given conditions, an assumption that might be made if the gas were being used in the metabolism of this organism.

Experiment IV. February 11, 1919. An emulsion of freshly precipitated and washed calcium carbonate was made in the inoculating material, and twelve glucose tubes with a reaction of pH = 8.2 were then inoculated with it. Six of these were incubated in the air-jar and six in the CO_2-jar. At the end of twenty hours the tubes in the CO_2-jar all had a very satisfactory growth of meningococcus whereas the tubes in the air-jar remained sterile. Apparently the insoluble calcium carbonate cannot bring about the satisfactory condition that is produced by the carbon dioxide. This is what would be expected if it is a matter of adjusting the initial reaction.

Experiment V. February 11, 1919. Twelve glucose blood agar slants with a reaction of pH = 8.2 were inoculated in the usual manner. Six of these were incubated in the CO_2-jar and six in a similar jar in which 10 per cent of the atmosphere was replaced by nitrogen gas. This was generated from sodium nitrite and ammonium chloride, washed by passage through a solution of sulphuric acid and one of sodium hydroxide and finally by shaking with a solution of sodium hydroxide. After twenty hours incubation the tubes in the CO_2-jar had a good growth while the tubes in the N_2-jar showed no growth. This is evidence that the carbon dioxide does not produce its effect by diminishing the oxygen tension, with the reaction of media here used. In an experiment given below it will be shown that with more acid reactions, i.e., the optimum for air growths, this gas has an inhibiting effect.

Experiment VI. February 18, 1919. An attempt was made to incorporate the carbon dioxide in the media by means of sodium bicarbonate. In each of six bottles was put 100 cc. of glucose-free agar with a reaction of pH = 7.6. After sterilization 2 cc., 4 cc., 6 cc., 8 cc., and 10 cc. of twice normal sodium bicarbonate was added respectively to five of these bottles, the sixth being used for a control. The addition of 2 cc. of twice normal sodium bicarbonate to 100 cc. of this agar gave it a reaction of pH = 7.9. The reaction of the other lots was not determined. Six tubes of each of these lots of media, with human blood, were inoculated in the given way, three incubated in the air-jar and three in the CO_2-jar. After twenty hours all the tubes in the CO_2-jar had a luxuriant growth whereas in the air-jar only the control tubes, with no sodium bicarbonate, showed any growth and this was scanty. The blood in the tubes containing 6 cc. or more of sodium bicarbonate per 100 cc. of media became very much decolorized upon incubation in the air-jar but retained its bright color in the CO_2-jar where the high alkalinity was neutralized by the carbon dioxide. This fact, together with the results of the growth, is evidence that the organism could not tolerate the more alkaline media produced by the addition of the sodium bicarbonate unless this was neutralized by the carbon dioxide. The experiment was repeated the next day and similar results obtained.

Experiment VII. February 19, 1919. Upon seeing the results of experiment VI, it was decided to make a series of lots of media with a very wide range of reaction. Eight different reactions were made from a lot of agar with a reaction of pH = 7.2 as represented in table 3.

TABLE 3

AMOUNT PER BOTTLE	N/1 HCl ADDED	pH	N/1 NaOH ADDED
cc.	cc.		cc.
250	6.0	4.7	0
250	4.0	5.3	0
250	2.25	6.0	0
250	1.5	6.7	0
250	0	7.2	0
250	0	7.8	1.0
250	0	8.4	4.5
250	0	9.0	8.0

For the two most alkaline reactions there was considerable flocculation during sterilization and a resultant decrease in alkalinity. Consequently a portion of the normal solution of sodium hydroxide was

added after sterilization to bring back the desired reaction. Six tubes of each reaction were inoculated with a four day old brain medium culture of the meningococcus. Three were incubated in the air-jar and three in the CO_2-jar. The results are tabulated in table 4.

TABLE 4

	REACTION OF MEDIA							
	pH = 4.7	pH = 5.3	pH = 6.0	pH = 6.7	pH = 7.2	pH = 7.8	pH = 8.4	pH = 9.0
Growth in air-jar, 24 hours...	0	0	0	0	0	0	0	0
Growth in CO_2-jar, 24 hours..	0	0	0	0	++	++	++	++
Growth in air-jar, 48 hours...	0	0	0	0	0	0	0	0
Growth in CO_2-jar, 48 hours..	0	0	0	++	++++	++++	++++	++++

This experiment was repeated the next day, using the same culture for inoculation, which was then five days old, and similar results were obtained.

Experiment VIII. February 21, 1919. In this experiment six tubes of each of the reactions used in the previous experiment were inoculated with a twenty-four hour brain medium culture which was made from the five day old brain medium culture used in the previous experiment. Three tubes of each reaction were incubated in the air-jar and three in the CO_2-jar. The results are tabulated in table 5.

TABLE 5

	REACTION OF MEDIA							
	pH = 4.7	pH = 5.3	pH = 6.0	pH = 6.7	pH = 7.2	pH = 7.8	pH = 8.4	pH = 9.0
Growth in air-jar, 24 hours.....	0	0	0	+++	++	+	0	0
Growth in CO_2-jar, 24 hours.....	0	0	0	+	++++	+++++	+++++	+++++

It wil' be noticed that the growth in the air-jar for the reaction pH = 6.7 was decidedly better than that for the same reaction in the CO_2-jar; but for the reaction pH = 7.2 the reverse is true, and for the reaction pH = 7.8 the growth is much heavier in the CO_2-jar than in the air-jar. The results of this experiment, together with the previous ones, may be interpreted as follows. This organism is very susceptible

to the reaction of the medium. The optimum reaction is found in a medium in which the pH = 7.0 or thereabout. The tension of the carbon dioxide used tends to bring all reactions more alkaline than this down to this figure. As the organism grows it produces acids which displace an equivalent amount of carbon dioxide and therefore do not greatly increase the acidity. This also explains a phenomenon noticed throughout these experiments, i.e., the first growth appears at the upper end of the slant, where the medium is thin and therefore effected more quickly by the carbon dioxide; but the final growth is heaviest on the lower end of the slant. In the CO_2-jar the carbon dioxide apparently increases the acidity of an initial reaction of pH = 6.7 enough to exert an inhibiting effect. This serves as additional evidence that this gas exerts its effect entirely by adjusting the reaction of the medium. At any rate any other effect is overshadowed in this experiment. The fact that frequently growths were obtained in the CO_2-jar with the less viable inoculations, as will be shown in the experiments given below, when no growth was obtained in the air-jar with any of the reactions used may find its explanation in the closer adjustment of the reaction of the medium to the optimum by the carbon dioxide than was secured by titration.

Experiment IX. February 23, 1919. Six tubes each of glucose and of glucose-free blood agar, reaction of pH = 8.0, were inoculated in the given manner and half of each lot incubated in the air-jar and half in the CO_2-jar. In this case both jars were thoroughly dried. To replace 10 per cent of the atmosphere in the CO_2-jar one liter of carbon dioxide was run into the jar by means of a rubber tube under a tuft of cotton in the bottom to prevent too rapid diffusion and escape of the gas. The gas was measured by replacement with water. A similar tuft of dry cotton was placed in the bottom of the air-jar. At the end of twenty hours all the tubes in the CO_2-jar, both glucose and glucose-free had a luxuriant growth whereas in the air-jar only one tube showed any growth and it was very scanty. Here again no difference was noticeable in the growth on the glucose and the glucose-free media in the CO_2-jar. This experiment is evidence that the effect of the carbon dioxide is not a moisture effect.

In the last three experiments a number of the tubes were contaminated by a Gram positive bacillus, probably *B. subtilis*. This contamination was only in the butt of the tube and did not obscure the growth of meningococcus in the upper part of

the slant. Moreover, for every different reaction used, at least one of the three tubes was not contaminated. No difference in the density of the growth, or other evidence of symbiotic effect, could be noticed in the contaminated and the uncontaminated tubes of like reaction and otherwise similar conditions.

Experiment X. February 27, 1919. Another attempt was made to incorporate the carbon dioxide in the medium by means of sodium bicarbonate, beginning with a more acid reaction. Agar was made up to a reaction of pH 6.5, and 100 cc. put into each of six bottles. The pH after sterilization was 6.8. One bottle was used as a control, and to the other five was added respectively, just before pouring the slants at a temperature of about 45°C., 0.2, 0.4, 0.8, 1.5, 2 grams of sodium bicarbonate. Even the addition of 0.2 gram gave a slight evolution of gas at this reaction and the alkalinity therefore increased to a pH of about 7.2. Six tubes of each of the different lots of media just described, containing the given amount of defibrinated human blood, were inoculated as before with a three day old brain media culture, and six with a twenty-four hour culture. The results after eighteen hours incubation are given in table 6.

TABLE 6

	Grams of $NaHCO_3$ per 100 cc. media					
	Control	0.2	0.4	0.8	1.5	2.0
Results from three day old culture						
Growth in air-jar	0	0	0	0	0	0
Growth in CO_2-jar	0	+	+++	+++	0	0
Results from one day old culture						
Growth in air-jar	+++	0	0	0	0	0
Growth in CO_2-jar	+	++++	++++	+++	0	0

The results of this experiment bear out the statement above that vigorous organisms grow in an atmosphere of air within a narrow range of reaction; the acidity of the optimum reaction in air is increased by the carbon dioxide used sufficiently to inhibit the growth; with more alkaline reactions this gas produces a condition which is much more favorable and will give growth with the less viable organisms which will no longer grow under the conditions used without the carbon dioxide.

Experiment XI. March 1, 1919. The various reactions of media described in table 3, in which the contamination occurred, were again inoculated, six tubes of each reaction with a five day old culture, six with a three day old culture, and six with a one day old culture. The results after twenty hours incubation are given in table 7.

TABLE 7

	REACTION OF MEDIA							
	pH = 4.7	pH = 5.3	pH = 6.0	pH = 6.7	pH = 7.2	pH = 7.8	pH = 8.4	pH = 9.0
Results from five day old culture								
Growth in air-jar	0	0	0	0	0	0	0	0
Growth in CO_2-jar	0	0	0	0	+	++	+++	+
Results from three day old culture								
Growth in air-jar	0	0	+	+++	++	+	0	0
Growth in CO_2-jar	0	0	0	+	++++	++++	+++	0
Results from one day old culture								
Growth in air-jar	0	0	+	+++	++	+	0	0
Growth in CO_2-jar	0	0	0	++	+++	++++	++++	+++

It will be noticed that in the above experiment this strain of meningococcus shows an inclination to grow within a wider range of reaction than in earlier experiments. Even the three day old culture gave a very satisfactory growth in air although not as abundant as in the CO_2-jar. The impression that one received from working with this organism was that in the later experiments it gave a more vigorous growth than in the earlier ones. This was probably due to its becoming accustomed to the artificial media. However the growth obtained above with the one day old inoculating material was distinctly more luxuriant than was obtained with the three day old material, a fact that could not be registered with the system of + signs.

When the above experiments were completed another strain of meningococcus was isolated in the laboratory from a case of cerebrospinal meningitis. Several experiments, similar to those described with the varying reactions of the media, were performed with it and this strain showed the same tendency, in as

striking a manner, to give better growth with the alkaline media in the CO_2-jar than was obtained in air with the most favorable reaction.

CONCLUSIONS

. Experiments were conducted with two strains of the meningococcus. The optimum reaction for twenty hour growths on 4 per cent defibrinated human blood agar lies between a pH of 6.7 and 7.4 when incubated in an atmosphere of air. Much better growths can be obtained by making the media with a pH lying between 7.6 and 8.4 and incubating in an atmosphere in which 10 per cent of the air is displaced by carbon dioxide. Frequently the less viable inoculations failed to grow in an atmosphere of air on any reaction of the media used, while a good growth was obtained if 10 per cent of the air in the atmosphere was replaced by carbon dioxide. This was explained by the closer adjustment of the reaction of the media to the optimum by means of the carbon dioxide and by the buffer effect of the equilibrium set up between the gaseous carbon dioxide and the carbonic acid and sodium bicarbonate in the medium. As the organism grows it produces acids which displace an equivalent amount of dissolved carbon dioxide and therefore the acidity is not greatly increased. It is possible that the principles here involved may be applied equally well to the growing of other delicate organisms. By making media with a pH of 7.8 to 8.0 and incubating the meningococcus in a partial atmosphere of carbon dioxide the same media may be used for this organism which are used for the pneumococcus and the streptococcus.[1]

[1] Since the above article was written a paper has appeared by Doctor Gates in the Journal of Experimental Medicine for April vol. xxix, Pp. 325, in which Doctor Gates has similarly shown that the effect of carbon dioxide on the growth of meningococcus is really due to its effect on the reaction on the medium. His experiments do not, however, bring out the fact that at times a more vigorous growth is obtained if the alkalinity of the medium is reduced to the optimum by carbon dioxide rather than by a mineral acid. Theoretically this is no more than would be expected, since there is an equilibrium between the carbon dioxide in the atmosphere and that in the solution.

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THE NOMENCLATURE OF THE ACTINOMYCETACEAE

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In a paper, recently published but written early in 1917 (Breed, Conn and Baker, 1918), the authors criticised some of the ideas underlying the report presented by the Committee on Characterization and Classification appointed by the Society of American Bacteriologists (1917). These criticisms were presented verbally at the 1917 meeting of the Society and as a result one of the authors (Breed) was asked to join the Committee in its work. Soon afterward the task was assigned to him of determining the type species for the genera Pseudomonas, Actinomyces and Nocardia. As the authors believe that such types ought not to be selected arbitrarily, a search of the literature has been made which has brought out many facts of interest. Certain of the conclusions reached are presented in the following paper, which will be followed by another on the nomenclature of the Pseudomonadaceae.

GENERIC TERMS USED IN THE ACTINOMYCETACEAE.

The Committee referred to has recommended that two genera be recognized in this family; Actinomyces, with *A. bovis* Harz as the type species, and Nocardia, with no type species named. The former genus is intended to include the anaerobic pathogens while the second is to include aerobic species, primarily saprophytic in nature.

Every student of the group realizes that the status of these terms has never been well established and that there is great diversity in usage. The confusion which exists has made it very difficult to get at the facts and has necessitated the study

of a large number of papers some of which have proved to be rare and not obtainable in any American libraries. While the references in the following pages have been made with care, it is scarcely possible that all mistakes have been avoided, or that all significant articles have been found. The authors would therefore greatly appreciate the correction of any mistakes which others may discover.

The first organism of this family which was described and named was *Streptothrix foersteri* Cohn, 1875, p. 186. It is a parasitic species occurring in concretions found in the human tear duct. While Cohn's figure of this species and his original description make it clear that his organism was one showing true branching of its filaments, yet Cohn himself did not feel entirely sure of the matter as is shown by the fact that on p. 204 he includes Streptothrix (followed by a question mark) with Cladothrix under "Zellfaden durch falsche Astbildung verzweigt." The type of branching found in Cladothrix was stated correctly by Cohn as false, but was confused by Cienkowsky (1877) who reports that at times the branches of Cladothrix seem to arise by true, not false, branching.

In view of this uncertainty it is not surprising to find that Winter (1879) actually included *Streptothrix foersteri* under the genus Cladothrix, stating in his characterization of the genus that all species in the genus show false branching. This unfortunate mistake appears again and again in the literature of the following decade and was greatly furthered by the confusing mistakes which Macé made. This author in 1888 described a true streptothrix under the name *Cladothrix dichotoma*. Then he later copied Cohn's figure of *Streptothrix foersteri* into the first edition (1889) of his text book under the caption *Cladothrix dichotoma*. In the second edition (1891), this error was continued by substituting a drawing of his own of a true streptothrix still under the caption *Cladothrix dichotoma*. In the third edition of his book (1897) he continues to use the generic term Cladothrix in place of Streptothrix Cohn and defends this usage on the ground that Cohn was not justified in making two genera for the species which he described under the names

Streptothrix foersteri and *Cladothrix dichotoma*, and that since Streptothrix was preempted by Corda, Cladothrix remains as the earliest valid name for the genus.

Other authors and especially those of recent years are however practically all in agreement that the filamentous thread bacteria with false branching which occur in fresh water should be placed in the genus Cladothrix Cohn or the genus Sphaerotilus Kützing (1833) entirely separate from those organisms which have been properly placed in the genus Streptothrix Cohn.

Migula's (1895) acceptance of the union of the genera Cladothrix, Streptothrix and Actinomyces in Engler and Prantl's Die Natürlichen Pflanzenfamilien under the term Cladothrix is later repudiated in his System der Bakterien (Migula 1900) where he uses Sphaerotilus in place of Cladothrix and excludes the species included under Streptothrix as belonging to the true fungi. However, Engler (1907), apparently without realizing this, accepts Migula's earlier conclusion and even makes the original mistake worse by using the combination *Sphaerotilus bovis* (Harz) Engler and implying that all other Actinomycetaceae should be placed in Sphaerotilus.

The second organism of this family which was found and described was the organism causing lumpy jaw (actinomycosis) of cattle. This was described in 1877 by Harz at Bollinger's request in a paper read before a scientific society in Munich. In publication however the name given by Harz to this organism (*Actinomyces bovis*) appears first in a paper written by Bollinger (1877, p. 485) where credit is given to Harz for having suggested the name. The following year, before the publication of the description of the species by Harz, Rivolta (1878) used the name *A. bovis* Harz in the title of his paper; but later in the body of his paper (p. 208) changed the name for trivial reasons to *Discomyces bovis*. The description written by Harz appeared in 1879 at which time Harz took exception in an appendix (p. 140) to the change of name made by Rivolta thereby showing clearly that Rivolta's paper was already in print. Later Rivolta (1884) announced himself as satisfied to retain the name Actinomyces proposed by Harz, both he, and also Harz, being ignorant at the

time that the generic term Actinomyce had already been used by Meyen (1827) for one of the higher fungi.

The confusion was increased by Afanasiev (1888), who claimed that *A. bovis* should be united with the genus Cladothrix as then known. Nevertheless in a footnote given on p. 84 of the German edition of this paper he remarks in a casual manner that one should use the name *Bacterium actinocladothrix* for this species, and this combination also appears in a German review of this article by Dittrich (1888). In a later paper read by Afanasiev and Schultz (1889) before the Third Congress of Russian Physicians in St. Petersburg, the original of which was printed in Russian, these authors use the term Actinocladothrix as if it were a generic name of equal rank with Cladothrix; and in at least two of the German reviews of this paper (Afanasiev and Schultz, 1889a and 1889b) Actinocladothrix is likewise used as if it were the name of a genus. It is therefore not surprising to find that Gasperini (1892, p. 183) and several later authors attribute the combination *Actinocladothrix bovis* to Afanasiev or to Afanasiev and Schultz. Apparently however this combination was never actually used by them.

Almost at the same time, Trevisan (1889) recognized that *Streptothrix foersteri* Cohn and *Actinomyces bovis* Harz were closely related and he placed both in a new genus Nocardia, distinct from Cladothrix. Unfortunately in doing this he continued Winter's mistake and described both Nocardia and Cladothrix as showing false branching. The new generic term was given on the ground that Streptothrix had been preempted by Corda (1839, p. 27) for another genus of fungi and that Actinomyce had been used by Meyen (1827). While Trevisan knew of the term Discomyces Rivolta as shown by the fact that he quotes *D. bovis* in his list of synonyms, he ignores its claim for recognition. In the same list of synonyms he credits the combination *Bacterium actinocladothrix* to Afanasiev. The new name, Nocardia, is given in honor of Nocard and it is evident from the fact that Trevisan places the species described by Nocard (1888) as the "bacille du farcin" as the first species in the genus under the name *N. farcinica* that he wished to make this species the type of the genus.

Sauvageau and Radais (1892) placed *Streptothrix foersteri* Cohn, *Actinomyces bovis* Harz, *Nocardia farcinica* Trevisan and other related species in the genus Oospora Wallroth (1833, p. 182) claiming that they were similar to the species already included in this genus. Likewise Thaxter (1891, p. 159) in describing the organism causing potato scab (now known to be very similar to the three species named above and usually placed in the same genus with them) named it *Oospora scabies*. However, further investigations have shown this combination of genera suggested by Sauvageau and Radais to be an unnatural one and it has not been generally accepted.

The status of the generic term, Carterii, proposed by Musgrave, Clegg and Polk (1908, p. 470) is sufficiently explained for all systematists by merely quoting their statement. After accepting Streptothrix as the term which they use for the pathogenic actinomycetes studied by them, they add the following:

> In making this decision, we are fully aware of the rights of those who favor *Actinomyces* or *Nocardia*, and under the circumstances are tempted to introduce a new name (*Carterii*) for the genus, together with a full and complete description.

A name proposed in this way is merely a nuisance to all who wish to see biological nomenclature placed on a stable footing.

Thus eight different generic terms (Streptothrix, Cladothrix, Sphaerotilus, Actinomyces, Discomyces, Actinocladothrix, Nocardia and Oospora) have been more or less generally used for the group of species under discussion; and confusion has increased as the years have passed because increasing knowledge has shown that the species on which these genera were founded were inadequately described by their authors.

Reference to Cohn's original description of *S. foersteri* shows that the only possible way in which this species can be recognized is in case it is shown that its habitat (concretions in the human tear duct) is sufficiently characteristic to identify it. The drawings given and the descriptions of morphology would apply equally well to any other species in this genus.

The situation is even worse in regard to *A. bovis* Harz for a dispute exists in regard to the nature of this organism. Some following the lead of Boström (1891) contend that it is an aerobic organism. Others have even regarded it as similar to, if not identical with certain common aerobic soil forms. On the other hand, Wolff and Isreal (1891) and later Wright (1905) describe the organism causing actinomycosis as an anaerobe, and Wright goes so far as to regard it as sufficiently distinct from the soil forms to place it in a genus separate from them. His view is nevertheless disputed recently by Pinoy (1913) who contends that there are two types of actinomycosis caused by different organisms and that the specific name "*bovis*" should be retained for the aerobic organism.

A very similar situation exists in regard to the organism causing potato scab (*Oospora scabies* Thaxter). That this organism belongs to the Actinomycetaceae is well established today; but Lutman and Cunningham (1914) have recently claimed, without reporting any inoculation experiments, that it is identical with *Actinomyces chromogenus* Gasperini, a common soil form. Krainsky (1914) has shown however that by the use of proper synthetic media, this "species" of Gasperini's may be separated into several distinct types; and the more recent work of Waksman and Curtis (1916), Drechsler (1919) and of one of us (Conn, 1917) has revealed the existence of many more distinct species. While all of the soil forms appear to be more closely related to the potato scab organism than to the forms causing actinomycosis and farcy, only a few of them agree with the potato scab organism even in cultural characteristics, and for these few, conclusive proof that they can cause potato scab (i.e., inoculation experiments) is still lacking.

When the cultural studies made by Krainsky, Waksman and Curtis, and one of us (Conn) are viewed in the light of the morphological studies of these species made by Lachnar-Sandoval (1898), Neukirch (1903) and more recently by Drechsler (1919), it becomes increasingly probable that future investigators will distinguish many species in the group and that they will find them as distinct and as limited in their distribution to specific habitats

as are other species of fungi. While the animal pathogens are better known today than are the soil saphrophytes, and very properly serve as type species for genera, they apparently represent but a small and highly specialized portion of the group of species included in the family Actinomycetaceae.

SUGGESTED SUBDIVISIONS OF THE GENUS ACTINOMYCES

Wright (1905) was apparently the first to suggest the subdivision of the genus Actinomyces Harz. This he does in such a way as to retain the name Actinomyces for the anaerobic organisms which he studied and which he regards as the true *Actinomyces bovis* Harz. However, apparently without having made any special study of the organisms concerned, he accepts the view that the organism causing bovine farcy (*Nocardia farcinica*) is closely similar to the soil saprophytes and suggests that the generic term, Nocardia, be used as an omnibus term to include all species except the anaerobic *A. bovis* and such other anaerobes as may later be identified. This view may be commended for the simple way in which it disposes of all species of no particular interest to pathologists; but it will hardly commend itself to systematists.

Pinoy (1913) disregarding the previous subdivision by Wright also subdivides the genus Actinomyces (for which he uses the term Nocardia). Since he finds that, while the more common type of actinomycosis is caused by an anaerobic organism, a less common type is caused by an aerobic species of the type described by Boström (1891), he regards *Nocardia bovis* (Harz) Blanchard 1896, p. 857, as an aerobic species. On the other hand he places the anaerobic organism described by Wolff and Isreal (1891) and by Wright (1905) in a new genus Cohnistreptothrix (p. 931). As a specific term he uses *isreali*, the specific term proposed by Kruse (1896, p. 56) for the organism described by Wolff and Isreal (1891). Pinoy likewise includes Cohn's original species in this new genus and from the form of the new generic term evidently regards *Cohnistreptothrix foersteri* (Cohn) as the type species of this new genus.

Discussion

Much of this confusion has arisen needlessly and because of ignorance of generally accepted principles of nomenclature. It offers a strong argument in favor of a general study of these principles by pathologists and bacteriologists. American bacteriologists having accepted the International Rules of Botanical Nomenclature (1905 and 1910) as a guide, this code will ordinarily be referred to in the following discussion. Inasmuch however as this code makes no reference to the selection of type species, reference will at times be made to the International Rules of Zoological Nomenclature (1915) in which specific rules governing the selection of type species are given.

Under both of these codes the original and frequently used term Streptothrix Cohn must be rejected because of the preemption of the term by Corda (1839) for an entirely different genus of fungi. Corda's term is moreover in common usage among mycologists today (see for example Stevens, 1913, p. 599). Even the most ardent advocate of the establishment of the validity of scientific names through usage will scarcely contend that Streptothrix Cohn should be retained when the list of species included in Streptothrix Corda is examined[1] and it is realized that new species have been placed in this genus as lately as 1914 and that specimens of these fungi are included in such a widely distributed and well known herbarium as that of the North American Fungi of Ellis and Everhart. The necessity for discarding Streptothrix Cohn is doubly emphasized by such an error as that of Stevens (1913, p. 599) who places *S. dassonvillei* Brocq-Rousseau (1907) under Streptothrix Corda although

[1] Genus Streptothrix Corda, 1839. Corda included a single species, *S. fusca* in the genus. Others have added the following species:

S. abietina Peck (Original description Buffalo Soc. Nat. Hist. Bull. No. 1, p. 69, 1873. Also in 25 Ann. Rept. N. Y. State Mus. Nat. Hist., p. 93, 1873).

S. atra Berkeley and Curtis (Grevillea, **3**, 107, 1875).

S. glauca Ellis and Everhart (Jour. Mycol., **4**, 107, 1888).

S. cinerea Morgan (Jour. Cincinnati Soc. Nat. Hist., **17**, 44, 1895).

S. pereffusa Sumstine (Mycologia, **6**, 34, 1914).

the author of this species places it in the very different genus Streptothrix Cohn.

The situation in regard to the use of the term Actinomyce (not Actinomyces) by Meyen (1827) is not quite the same as that just discussed. As early as 1830, Meyen himself points out that his species *A. horkellii* is identical with *Tremella meteorica* Persoon; and so far as the authors of the present paper are aware this is the last appearance of Actinomyce Meyen in the literature except as a synonym or homonym. Under these conditions, it is not only possible but necessary under the International Botanical Rules (see Chap. III, Sect. 2, Art. 20 and Chap. III, Sect. 6, Art. 50) to establish the term Actinomyces Harz as a *genus conservandum* by action taken at an International Botanical Congress. Pending such action, it is to be hoped that botanists and bacteriologists will continue to use Actinomyces Harz. Such usage would be encouraged if action were taken by the Society of American Bacteriologists recommending that Actinomyces Harz, not Actinomyce Meyen, be recognized as a valid genus.

The only valid argument which can be brought against the use of Actinomyces is the one which is brought forward by those who believe in the strict application of the Law of Priority. For such, as ably explained by Blanchard (1900), the term which must be accepted is Discomyces Rivolta. Neither the fact that this term has an unfortunate resemblance to the commonly used Discomycetes nor the fact that Rivolta (1884, p. 183) himself, in ignorance of the true state of the case, accepted Harz's name in a later publication invalidates Discomyces. The fact however that Discomyces has fallen into almost complete disuse because of these things gives strong reason for not regarding priority in this case.

No one, so far as known, has attempted to establish the valid status of Actinocladothrix, while Blanchard (1900) has explained why Nocardia has no standing as a generic term for the entire group of organisms under discussion.

Oospora, Sphaerotilus and Cladothrix may be dismissed from consideration for, as already indicated, there is general agreement

that the organisms properly included under these terms should not be included in the same genus or even in the same family with the Actinomycetaceae.

Two attempts have already been made to establish the validity of a generic name for this group of organisms through legislative action both of which must be regarded as abortive. The first was taken by a Committee of the English Society of Pathologists who, as reported by Foulerton (1912, p. 304), approved the term Streptothrix Cohn in ignorance of the general and wide usage of Streptothrix Corda. The second was taken by the Botanical Section of the First International Congress of Comparative Pathology according to Pinoy (1913, p. 933) and confirmed by him in a letter dated September 9, 1918. The name accepted was Nocardia. The official record of the Congress however contains no reference to this action which is stated to have been taken during the discussion of a paper by Potron (1912). As this action ignores the stronger claims of Actinomyces and Discomyces, and does not appear in the official record, it cannot be regarded as final.

Nocardia Trevisan is valid only in case the species *N. farcinica* is placed in a genus distinct from *Actinomyces bovis*. The present justification for this separation turns upon the identity of *A. bovis*. If *A. bovis* is an aerobic species as described by Boström (see p. 590) it is apparently so similar to *N. farcinica* that there is scant justification for placing the two species in separate genera, and Nocardia must remain a synonym of Actinomyces. If however, the difference between these species is sufficient to justify separating the genera then Nocardia becomes a valid term with *N. farcinica* as the type species.

In our judgment neither Wright's nor Pinoy's investigations, based as they are on the study of the pathogens only, justify separating the genus into two parts. Future investigations may well show that there is a closer resemblance between the organism causing bovine farcy and the organism causing actinomycosis than between the farcy organism and soil saphrophytes. Attempts so far made to subdivide the genus appear to us as premature and certain to lead to further confusion in the nomenclature of the group.

TYPE SPECIES FOR THE GENERA STREPTOTHRIX, ACTINOMYCES, NOCARDIA AND COHNISTREPTOTHRIX

Much of the confusion in the use of these terms would have been avoided if investigators had observed the rules governing the selection of type species generally observed by systematists, and definitely formulated in the International Rules of Zoological Nomenclature, Article 30 (1915).[2] Many men have regarded the four terms quoted as exact synonyms when, as a matter of fact, since three different species are properly regarded as types of the four genera mentioned, they are exact synonyms only so long as these three species are included in the same genus. A brief discussion of the status of each of the three species with a list of synonyms follows:

Streptothrix, Cohn. In case this term is used, *S. foersteri* Cohn must be recognized as the type species as it was the only species named at the time the genus was proposed (mono-typical genus). This species has often been placed in other genera by writers who consider Streptothrix Cohn invalid, and some of these writers wrongly consider the early date at which this species was described to necessitate its acceptance as the type species of any genus which includes it. Thus Vuillemin (1913) incorrectly accepts it as the type species of Nocardia.

It is generally recognized that the type species of any genus must have been included in the genus at the time that it was originally described. For this reason *S. foersteri* cannot be regarded as the type in case the terms Actinomyces, Discomyces or Actinocladothrix are used. Trevisan did list *N. foersteri* (syn. *S. foersteri*) as one of the species of Nocardia in his original description of the genus; but he plainly indicated (1) by the name used for the genus and (2) by the fact that he placed *N. farcinica* as the first species in the genus that he regarded the latter species as the type of the genus. The right of an author

[2] After this was written it was discovered that the Committee on Generic Types of the Botanical Society of America have recommended practically the same rules as those used by the Zoologists (see the report of this Committee which has just appeared in Sci., N. S., **49**, 333–336. 1919.)

to fix the type of a new genus ought to be universally regarded, and is so regarded by all who observe generally accepted rules of nomenclature.

Synonomy

Streptothrix foersteri Cohn, 1875, p. 186.
Syn. Cladothrix foersteri (Cohn) Winter, 1879, p. 60.[3]
Nocardia foersteri (Cohn) Trevisan, 1889, p. 9.
Oospora foersteri (Cohn) Sauvageau and Radais, 1892, p. 252.
Actinomyces foersteri (Cohn) Gasperini, 1894, p. 684.
Discomyces foersteri (Cohn) Gedoelst, 1902, p. 176.[4]
Cohnistreptothrix foersteri (Cohn) Pinoy, 1913, p. 937.

Actinomyces. For those persons who recognize the validity of Actinomyces the matter of the type species is simple, so far as nomenclature is concerned, as *A. bovis* Harz was the only species named at the time the genus was named (monotypical genus). As already indicated however the identity of this species is far from being satisfactorily established.

If any are inclined to recognize the validity of Discomyces or Actinocladothrix, the same species must serve as type and for the same reason.

Synonomy

Actinomyces bovis Harz (see Bollinger, 1877, p. 485).[5]
Syn. Discomyces bovis (Harz) Rivolta, 1878, p. 208.[6]
Bacterium actinocladothrix Afanasiev, 1888, p. 84.[7]

[3] The date given (1879) is the date when the manuscript was completed. The completed volume in which this combination appears was not published until 1884, but from the fact that Zopf (1882, p. 13) refers to Winter it is evident that the portion of the volume in which Cladothrix is described, was published earlier than 1882. Because of the question in regard to the date of publication, this combination is frequently incorrectly attributed to Zopf, Hueppe or even Schroeter.

[4] This combination is attributed to Blanchard by Vuillemin (1913) without reference to the place of publication but search has thus far failed to show that he has ever used this combination.

[5] Sometimes incorrectly ascribed to Bollinger.

[6] Sometimes ascribed to Rivolta and Micellone (1879).

[7] The combination Actinocladothrix bovis is frequently incorrectly ascribed to Afanasiev or to Afanasiev and Schultz.

Nocardia actinomyces Trevisan, 1889, p. 9.
Streptothrix actinomyces (Trevisan) Rossi-Doria, 1891, p. 405.
Cladothrix bovis (Harz) Macé, 1891, p. 666.[8]
Oospora bovis (Harz) Sauvageau and Radais, 1892, p. 271.
?Actinomyces bovis sulfureus Gasperini, 1894, p. 684.
Nocardia bovis (Harz) Blanchard, 1896, p. 857.
Cladothrix actinomyces (Trevisan) Macé, 1897, p. 1038.
Streptothrix actinomycotica Foulerton, 1899, p. 780.
Streptothrix bovis communis Foulerton, 1901, p. 50.
Streptothrix bovis (Harz) Chester, 1901, p. 361.[9]
Sphaerotilus bovis (Harz) Engler, 1907, p. 5.

To this list must also be added, *Streptothrix isreali* Kruse, 1896, p. 56, *Actinomyces isreali* (Kruse) Lachnar-Sandoval 1898, p. 64, *Discomyces isreali* (Kruse) Gedoelst, 1902 p. 163 and *Cohnistreptothrix isreali* (Kruse) Pinoy, 1913, p. 931, in case investigations establish the fact that there is but one organism causing bovine actinomycosis.

Nocardia. This genus was first described in a paper (Trevisan, 1888) which is now very rare and apparently unobtainable in American libraries. The examination of a copy seen through the courtesy of Prof. C. Gorini of Milan shows that DeToni and Trevisan (1889) copied the portion of this paper describing this genus with little, if any, change. In the original paper, as in the paper by DeToni and Trevisan, five species are given in the genus, the first of these being *N. farcinica* Trevisan, the species described, but not named, by Nocard (1888). While this species is not definitely named as the type species, there is not the slightest question but that Trevisan regarded it as the type species of the new genus. *N. actinomyces* Trevisan (Syn. *Actinomyces bovis* Harz) is given as the second species followed by *N. foersteri* (Cohn) Trevisan (Syn. *Streptothrix foersteri* Cohn).

The species *N. farcinica* must therefore stand as the type species if the term Nocardia is used no matter what limits are set for the genus.

[8] Sometimes incorrectly ascribed to Migula.

[9] This combination is given as a synonym by Foulerton (1901) and it is possible that others used it before Chester.

Synonomy

Nocardia farcinica Trevisan, 1889, p. 9.[10]
Syn. Streptothrix farcinica (Trevisan) Rossi-Doria, 1891, p. 405.
Actinomyces farcinicus (Trevisan) Gasperini, 1892a, p. 222.
Oospora farcinica (Trevisan) Sauvageau and Radais, 1892, p. 248.
Actinomyces bovis farcinicus Gasperini, 1894, p. 684.
Cladothrix farcinica (Trevisan) Macé, 1897, p. 1047.
Streptothrix farcini bovis Kitt, 1899, p. 511.
Streptothrix nocardii Foulerton, 1901, p. 51.[11]
Discomyces farcinicus (Trevisan) Gedoelst, 1902, p. 167.
Actinomyces nocardii (Foulerton) Buchanan, 1911, p. 378.

The combination *Bacillus farcinicus* apparently appears first in the literature as a synonym in a list given by Gasperini, 1892a, p. 183 where it is attributed to Nocard. Nocard appears, however, to have always used the expression "bacille du farcin," and never to have given a Latin name to this organism.

Cohnistreptothrix. Pinoy (1913) has named two species in this genus, *C. foersteri* and *C. isreali*, neither of which are specified as the type species of the genus. It is evident however from the form of the generic name that he wishes *C. foersteri* to be recognized as the type species and it should be so recognized by those who accept his subdivision of the genus.

SUMMARY

1. Because of confusion between Streptothrix Corda 1839 and Streptothrix Cohn 1875 and the general use of the former term by mycologists, the latter term should be generally disregarded. According to the International Rules of Botanical Nomenclature, the limited use of the term Actinomyce by Meyen in 1828 and 1832 is not sufficient to invalidate the generally used Actinomyces Harz 1877 provided the latter is accepted as a *genus conservandum* by an International Botanical Congress. The continued use of the latter term is therefore recommended. The type species of the genus is *A. bovis* Harz.

[10] Frequently incorrectly attributed to DeToni and Trevisan.
[11] Incorrectly ascribed to Nocard by Foulerton.

2. The generic terms Discomyces Rivolta and Actinocladothrix Afanasiev and Schultz are and must remain synonyms of Actinomyces for all those who recognize the right of the International Botanical Congress to establish the validity of botanical names through legislative action. Those that contend that strict priority should govern the matter are apparently limited in their choice to the little used and confusing term Discomyces, a term repudiated even by its author. Oospora Wallroth, Sphaerotilus Kützing and Cladothrix Cohn do not properly apply to the organisms discussed in this paper.

3. There appears to be no justification for the use of the term Nocardia Trevisan for the entire group of organisms included in the Actinomycetaceae. It may however be properly used for a subdivision of the genus Actinomyces, provided however *N. farcinica* is retained in the genus Nocardia and is established as the type of the genus.

4. Knowledge of the group is however so imperfect that neither the subdivision of the group proposed by Wright (and accepted by the Committee on Classification and Characterization of the Society of American Bacteriologists) nor that proposed by Pinoy can be regarded as satisfactory.

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A CULTURE MEDIUM FOR THE MAINTENANCE OF STOCK CULTURES OF BACTERIA

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The magnitude of the question of the maintenance of stock cultures was brought to my attention by the work in the culture bureau at Parke, Davis and Company. Frequent transfers mean an immense amount of labor and material, for some cultures such as the meningococcus and gonococcus will live only about ten days even on special media such as ascitic agar. This means they must be transferred at least twice a week to be sure of keeping them viable and characteristic. *B. pertussis* and *B. influenzae* require weekly transfer and there are very few organisms that are safe to leave more than a month between transfers. It is evident, therefore, that the maintenance of cultures with the least amount of labor and material, so that all characteristic properties shall remain the same, is of interest and importance to those who are attempting to carry cultures in great number and variety.

Various media have been tried for special organisms, with considerable success, but a special medium for each different culture is a disadvantage in the smaller laboratories where media making is not carried on as a specialized art. In this work an attempt has been made to obtain some medium, as simple as possible, upon which various micro-organisms will remain viable and characteristic for a long time. While the work is by no means complete, it has been carried far enough and with enough success, to justify a report and a trial on a larger scale.

Drying has a tendency to attenuate or kill the majority of organisms, while on the other hand cultures in liquid media are rather difficult to handle and in many cases form disintegration

and by-products which ultimately become detrimental to the organisms themselves. Experiment has shown that mechanical protection is of value in the preserving of plant seeds and with this idea in mind, gelatin media of various acidities and contents were tested for their preserving qualities on organisms of various kinds.

Freshly inoculated gelatin cultures were incubated at 37°C., at which temperature the gelatin became liquid thus allowing the organisms to multiply and spread throughout the tube, forming a homogeneous mixture in twenty-four to forty-eight hours. Then these cultures were stored at room temperature, except the meningococcus and gonococcus which were kept at 37°C. The gelatin at room temperature partially solidified thus forming more or less of a mechanical protection for the organisms. Transfers were made periodically upon a suitable medium and special tests were made to see that the organisms had lost none of their characteristic properties, and as a control transfers were made from the same strains which had been kept by the regular method of frequent transfer upon suitable media.

The gonococcus and the meningococcus, *B. typhosus*, *B. paratyphosus* and *B. coli* were the organisms under most extensive and detailed study. *B. dysenteriae*, *B. diphtheriae*, *B. pseudodiphtheriae*, *B. pertussis*, *Micrococcus catarrhalis*, the Staphylococcus, and *Streptococcus viridans*, *S. fecalis* and *S. pyogenes* were also put under test.

The media under investigation were: I, regular gelatin, made according to standard methods, 1 per cent acid; II, regular gelatin plus 2 per cent glucose; III, regular gelatin 0.4 per cent acid; IV, regular gelatin 1 per cent acid plus 2 per cent glucose plus calcium carbonate; V, regular gelatin 1 per cent acid plus 2 per cent glucose plus rabbit liver.

A few months demonstrated that the more complex media had little or no advantage over the regular nutrient gelatin 1 per cent acid. The *B. typhosus*, *B. paratyphosus*, *B. coli*, *B. dysenteriae* and the meningococcus and gonococcus showed, if anything a preference for the 1 per cent acid gelatin. *B. pertussis* was not living at the end of one month on the regular gelatin plus 2 per

TABLE 1

Comparison of agglutinability of three strains of B. typhosus maintained for three months in gelatin and on plain agar

CULTURES	DILUTIONS OF ANTITYPHOID SERUM (RABBIT)							
	1-500	1-1000	1-2000	1-2500	1-3000	1-3500	1-4000	1-5000
No. 1 Sp..	+++	−	++	++	−	+	−	−
No. 1 R..	+++	−	+	+	−	+?	−	−
No. 2 Sp..	+++	+++	+++	+++	+++	+++	+++	++
No. 2 R...	+++	+++	+++	++	++	++	++	+
No. 3 Sp..	+++	+++	+++	+++	+++	+++	+++	+++
No. 3 R...	+++	+++	+++	+++	+++	+++	+++	++

Sp = Cultures maintained in 1 per cent acid gelatin without transfer.

R = Cultures maintained in the regular manner, by transfers every three weeks on agar. *B. coli* was handled in the same way.

TABLE 2

Comparison of agglutinability of six strains of B. coli maintained for three months in gelatin and on plain agar

CULTURES	DILUTIONS OF WHITE SCOURS SERUM							
	1-500	1-1000	1-2000	1-4000	1-8000	1-16000	1-32000	CONTROL
No. 4 Sp..	+++	+++	+++	+++	+++	++	+	−
No. 4 R...	+++	+++	+++	+++	+++	++	+	−
No. 5 Sp..	+++	+++	+++	+++	++	+	−	−
No. 5 R...	+++	+++	+++	+++	++	+	−	−
No. 6 Sp..	−	−	−	−	−	−	−	−
No. 6 R...	−	−	−	−	−	−	−	−
No. 7 Sp..	+++	+++	+++	+++	++	+?	−	−
No. 7 R...	+++	+++	+++	+++	+?	−	−	−
No. 8 Sp..	+++	+++	+++	+++	+++	+++	++	−
No. 8 R...	+++	+++	+++	+++	+++	+++	++	−
No. 9 Sp..	++++	+++	+++	+++	+++	+++	++	−
No. 9 R...	+++	+++	+++	+++	+++	+++	++	−

cent glucose, while from the plain gelatins 1 per cent acid and 0.4 per cent acid good growths were obtained. Transfers from the eight months old cultures of *B. pertussis* in 1 per cent acid gelatin grew abundantly but with some involution forms; two months was about the limit of growth for *B. pertussis* on the 0.4 per cent acid gelatin.

Micrococcus catarrhalis gave an abundant growth upon transfer from both the 0.4 per cent acid gelatin and the 1 per cent acid gelatin plus 2 per cent glucose after three months.

Streptococcus viridans, at the end of one month, seemed in approximately the same condition on media I–II and III, while at the end of two months the cultures on the 0.4 per cent and 1 per cent acid gelatin seemed to have obtained the lead.

Hence most of the work from this point was carried along on 1 per cent acid gelatin.

At the end of three months, transfers were made onto plain agar slants from the gelatin cultures of *B. typhosus* nos. 2, and 3 and also from the corresponding cultures which had been maintained in the regular manner by transfer every three weeks on plain agar. The final transfer onto plain agar was not for rejuvenation but merely a means of getting the culture into a proper suspension for the agglutination test. At the end of twenty-four hours suspensions were made of these subcultures and the agglutination test gave the results shown in tables 1 and 2.

At the end of eight months *B. typhosus* nos. 2 and 3 were growing well, were microscopically characteristic and the agglutination tests were as shown in table 3.

TABLE 3

Comparison of agglutinability of two strains of B. typhosus maintained for eight months in gelatin and on plain agar

CULTURES	DILUTIONS OF ANTITYPHOID SERUM (RABBIT)						
	1-200	1-1000	1-1600	1-2000	1-3000	1-4000	Control
No. 2 Sp	+++	++	+	−	−	−	−
No. 2 R	+++	+++	+	−	−	−	−
No. 3 Sp	+++	+++	+	−	−	−	−
No. 3 R	++++	+++	+	−	−	−	−

At the end of eleven months the *B. coli* were growing well and were microscopically characteristic. The agglutination tests were as shown in table 4.

TABLE 4

Comparison of agglutinability of three strains of B. coli maintained for eleven months in gelatin and on plain agar

CULTURES	DILUTIONS OF WHITE SCOURS SERUM						
	1-200	1-2000	1-10,000	1-20,000	1-24,000	1-30,000	Control
No. 4 Sp.	++++	+++	+	−	−	−	−
No. 4 R.	++++	+++	+?	+	+	−	−
No. 8 Sp.	++++	+++	+++	++	++	−	−
No. 8 R.	++++	+++	+++	++	++	−	−
No. 9 Sp.	++++	++++	+++	+++	+?	−	−
No. 9 R.	++++	++++	+++	+++?	+?	−	−

The meningococcus and gonococcus were planted on 1 per cent acid gelatin, stored at 37°C. and tested at the end of three months for viability, microscopical appearance and serum reaction. All cultures were found in first class condition. At the end of seven months these cultures were again tested, good growths were obtained, of characteristic microscopical appearance, and the serum reactions were as shown in table 5.

TABLE 5

Comparison of agglutinability of one strain of meningococcus maintained for seven months in gelatin and on ascitic agar

CULTURES	DILUTIONS OF ANTIMENINGOCOCCIC SERUM						
	1-20	1-50	1-100	1-200	1-400	1-600	Control
No. 10 Sp.	+++	+++	+++	++	+	?	−
No. 10 R.	+++	+++	+++	++	+	?	−

TABLE 6

Comparison of agglutinability of four strains of meningococcus maintained for eight months in gelatin and on ascitic agar

CULTURES	DILUTIONS OF ANTIMENINGOCOCCIC SERUM						
	1-50	1-200	1-400	1-600	1-800	1-1000	Control
No. 11 Sp.	++++	++++	+++	+++	+?	−	−
No. 11 R.	++++	++++	+++	+++	+?	−	−
No. 12 Sp.	++++	++++	+++	+++	+++	+++	−
No. 12 R.	++++	++++	+++	+++	+++	+	−
No. 13 Sp.	++++	++++	++++	++++	++++	+++	−
No. 13 R.	++++	++++	++++	++++	++++	+++	−
No. 14 Sp.	++++	++++	+++	+++	++	+	−
No. 14 R.	+++++	++++	+++	+++	++	+	−

At the end of eight months the meningococci were again tested and found growing well; they showed characteristic microscopical appearance and the serum reactions were as shown in table 6.

SUMMARY

A medium of the following composition has been found favorable for the preservation of *B. typhosus*, *B. paratyphosus*, *B. coli*, *B. dysenteriae*, *B. pertussis*, *Micrococcus catarrhalis*, a streptococcus and the meningococcus and gonococcus for several months without transfer:

Nutrient Gelatin:
- Chopped beef ..... 500 grams
- Water ..... 1000 cc.

Heated in a water bath 50–55°C. for 1 hour.
Strained through bag cloth, volume restored:
- Peptone ..... 10 grams
- NaCl ..... 5 grams
- Gelatin ..... 100 grams

Dissolved, filtered, adjusted to 1 per cent acid.*
Sterilized for twenty minutes at 110°C.

Inoculation is made and the cultures are placed at 37°C. for twenty-four hours after which they are stored at 20°C. with the exception of the meningococcus and gonococcus which are kept at 37°C.

Without further attention *B. typhosus* retained its viability, characteristic appearance, and agglutinating power for eight months; *B. coli* for eleven months; meningococcus for eight months. The serum reactions were not determined on cultures except as reported. *B. paratyphosus*, *B. pertussis* and *B. dysenteriae* retained their viability and characteristic appearance for eight months. The streptococcus was still in good condition at the end of four months.

*In the continuation of this work in the laboratories of the Digestive Ferments Company, reactions are measured in terms of H-ion concentrations, which standard of measurement will be used in the future.

THE VALUE OF PRESUMPTIVE TESTS FOR BACILLUS COLI BASED ON THE ROUTINE USE OF LACTOSE BILE AND LACTOSE BROTH

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Previous to 1906, glucose broth was generally used in the presumptive test for *Bacillus coli*. As a result of the work of Jackson (1906, 1907), Weston and Tarbett (1907), Sawin (1907), and others (Hale and Melia, 1910), lactose peptone bile was substituted for glucose broth by the American Public Health Association in the 1912 Standard Methods of Water Analysis. Doubt was, however, thrown upon the accuracy of the results obtained by the use of lactose bile by Jordan (1913) and Obst (1916), which resulted in another change by the American Public Health Association in 1917, lactose broth being substituted for lactose bile. The authors cited showed that lactose bile not only inhibited organisms outside of the typhoid-colon group, but that it also had an inhibitive action on members of this group itself.

In this laboratory, both bile and broth are used for the detection of *B. coli* in tap waters; and the present study is based on examinations of 1899 samples of tap water showing fermentation in bile, or broth, or both, from the forty-six city surface supplies in Kansas, of which weekly tests are made. These cover the period from July 1, 1918, to May 15, 1919.

The bile used is prepared by diluting oxgall 50 per cent with distilled water and adding 1 per cent lactose and 1 per cent peptone. Both large and small culture tubes with inverted vial fermentation tubes are filled,—the large tubes with 15 cc. of medium and the small ones with 7 cc.

The broth, which is tubed in the large fermentation tubes, contains 0.3 per cent of Liebig's Beef Extract, 1 per cent peptone and 1 per cent lactose, and is adjusted to a point neutral to phenolphthalein.

Endo plates are made of 3 per cent agar with 1 per cent lactose and 0.37 per cent fuchsin decolorized with 5 per cent sodium sulphite. The reaction is adjusted to 0.5 per cent acid to phenolphthalein. This amount of fuchsin gives a rapid and brilliant coloration with acid formers, and a good metallic sheen on the colonies of *B. coli*.

Each sample is inoculated as follows: 1 cc. into each of five small bile tubes, 10 cc. into one large bile tube, 10 cc. into each of three large broth tubes.

These are incubated at 37° and after twenty-four hours, the cultures are read; those showing 10 per cent or more gas in the fermentation tube, or showing active fermentation, being streaked on Endo plates for confirmation, and the remaining cultures being returned to the incubator until forty-eight hours have elapsed. They are then read once more, positive cultures confirmed, and all discarded. Endo plates are incubated about twenty hours.

In recording results, twenty-four hour readings are marked in red ink, forty-eight hour readings in blue ink, with + and − to show presence or absence of gas. To indicate a 10 cc. sample, a circle is placed around the symbol. Bile results are always recorded above broth to avoid confusion.

Table 1 shows the relationship between confirmatory tests and the presence of gas in one or both of the bile and broth media. These results have been studied in an effort to learn if the simultaneous use of the two media might make confirmation unnecessary.

When both media are used and both show fermentation, 75.05 per cent confirm in either bile, broth, or both media. When bile alone shows fermentation, which is in a very small number of samples, 34.42 per cent confirm, as opposed to 25.20 per cent when broth alone is positive, which occurs in ten times as many cases. According to these results when both media are used in the presumptive test, there are fewer mistakes than when either is used separately.

TABLE 1

	NUMBER SAMPLES BILE AND BROTH FERMENTING	NUMBER SAMPLES BILE AND BROTH CONFIRMING	PER CENT SAMPLES BILE AND BROTH CONFIRMING	NUMBER SAMPLES BILE ONLY CONFIRMING	PER CENT SAMPLES BILE ONLY CONFIRMING	NUMBER SAMPLES BROTH ONLY CONFIRMING	PER CENT SAMPLES BROTH ONLY CONFIRMING	TOTAL PER CENT CONFIRMING
Bile, +... Broth, +.	1223	732	61.48	68	5.56	98	8.01	75.05
Bile, +... Broth, −	61			21	34.43			34.42
Bile, −... Broth, +.	615					155	25.20	25.20

The next step was to examine the results to determine if the number of broth tubes showing fermentation is any indication of the presence of typical *B. coli*.

It will be noted from table 2 that when all three broth tubes are positive, the percentage confirmed is above that calculated

TABLE 2

	NUMBER SAMPLES BILE AND BROTH FERMENTING	NUMBER SAMPLES BILE AND BROTH CONFIRMING	PER CENT SAMPLES BILE AND BROTH CONFIRMING	NUMBER SAMPLES BILE ONLY CONFIRMING	PER CENT SAMPLES BILE ONLY CONFIRMING	NUMBER SAMPLES BROTH ONLY CONFIRMING	PER CENT SAMPLES BROTH ONLY CONFIRMING	TOTAL PER CENT CONFIRMING
Bile, +... Broth, 3+	888	618	69.59	37	4.16	66	7.43	81.18
Bile, +... Broth, 2+	193	79	40.93	15	7.77	23	11.91	60.61
Bile, +... Broth, 1+	131	49	37.40	13	9.92	10	7.63	54.95
Bile, −... Broth, 3+	178					52	29.26	29.26
Bile, −... Broth, 2+	158					36	22.78	22.78
Bile, −... Broth, 1+	289					65	22.49	22.49

for the whole number of tests with both media fermenting, the average in the former being 81.18 per cent and in the latter 75.05 per cent. When only two of the three tubes are positive, 60.61 per cent confirm, and when only one ferments 54.95 per cent confirm. If both media are used and there is fermentation in any of the bile and all of the broth tubes, fewer unconfirmed presumptive tests will be obtained than in the cases where the number of tubes reacting positively is one or two.

TABLE 3

	NUMBER SAMPLES BILE AND BROTH FERMENTING	NUMBER SAMPLES BILE AND BROTH CONFIRMING	PER CENT SAMPLES BILE AND BROTH CONFIRMING	NUMBER SAMPLES BILE ONLY CONFIRMING	PER CENT SAMPLES BILE ONLY CONFIRMING	NUMBER SAMPLES BROTH ONLY CONFIRMING	PER CENT SAMPLES BROTH ONLY CONFIRMING	TOTAL PER CENT CONFIRMING
Bile, +.....								
Broth, +, 24 hours.	387	362	93.54	6	1.55	10	2.58	97.67
Bile, +, 24 hours...	8			3	37.50			37.50
Broth, −.....								
Bile, −.....								
Broth, +, 24 hours.	42					23	54.76	54.76
Bile, +, 24 hours...								
Broth, +, 48 hours	82	50	69.75	7	8.53	4	4.89	83.17
Bile, +, 48 hours..								
Broth, +, 24 hours	124	86	69.35	3	2.43	24	19.35	91.13

Another point for consideration is whether or not this same conclusion applies when the broth only is positive. By comparing the last three percentages in the preceding table this is seen to be the case, as when three tubes are positive there is 29.26 per cent confirmation, when two are positive, 22.78 per cent when only one is positive, 22.49 per cent, respectively.

A study of the time factor was next made and summarized in table 3.

There is no need for confirmation when both bile and broth show fermentation in twenty-four hours, as 97.67 per cent of

the samples so reacting proved to be positive in either bile, broth, or both media. If either bile or broth is positive in twenty-four hours and the other in forty-eight hours, there is a higher percentage of samples containing *B. coli* than when both bile and broth are positive only after forty-eight hours, as is shown by 83.17 per cent of confirmation with bile positive in twenty-four hours and broth in forty-eight hours, and 91.13 per cent with broth positive in twenty-four hours and bile in forty-eight hours.

This would indicate that while bile is fermented more slowly than broth, yet in dealing with contaminated waters it is just as accurate.

The statistics of these results on 1899 samples of surface waters examined for pollution, where both bile and broth are used simultaneously, would suggest the following conclusions:

1. If there is fermentation in both bile and broth tubes, the presumptive test is reliable in 75.05 per cent of all cases considered

2. If all three broth tubes, as well as the bile, are positive, a greater percentage of tests are confirmed as *B. coli* than is the case when only one or two tubes out of three are positive.

3. When broth alone is positive the water in 70 per cent to 78 per cent of cases is proved not to contain *B. coli*.

4. Samples with both media positive in twenty-four hours contain *B. coli* in 97.67 per cent of cases and therefore do not need confirmation.

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MORPHOLOGICAL CHANGES DURING THE GROWTH OF BACTERIA

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The life cycle of bacteria has been studied by a number of different methods. Some investigators (Müller, 1895, Lane-Claypon, 1909, Chick, 1912, Chesney, 1916, etc.) have determined the numbers of viable organisms which may be present at different periods following the transplantation of the organisms to fluid culture media. In such examinations, certain periods or phases of growth have been observed. Lane-Claypon recognized four phases in the life of a bacterial culture: (1) the latent period, or the initial phase of slow growth or no growth at all; (2) the period of maximum rate of growth, or logarithmic period, when the organisms are dividing regularly; (3) the stationary period in which the number of organisms remains more or less constant; (4) the period of decline when the numbers of living bacteria are diminishing. Buchanan (1918), analyzing the situation somewhat more closely, designates seven growth phases:

1. Initial stationary phase.

2. Positive growth acceleration phase or period of lag during which the average rate of increase in numbers of organisms increases with the time.

3. Logarithmic growth phase. During this phase the minimal average generation time and therefore the maximum rate of division is maintained throughout the period.

4. Phase of negative growth acceleration, during which the rate of division is decreased.

5. Maximum stationary phase during which there is practically no increase in the number of bacteria.

6. Phase of accelerated death. During this phase the numbers of viable bacteria are decreasing.

7. Logarithmic death phase in which the rate of death remains constant.

These phases of growth of a bacterial culture, however they may be designated, obviously merge into each other, the duration of each phase varying somewhat depending upon the organism and the different growth conditions.

Other observers have attempted to obtain information in regard to possible growth cycles among bacteria by studying the development of individual bacteria in hanging block and hanging drop cultures. Some interesting details of the simple cell division of bacteria have been observed, such as the demonstration of the division of the nuclear substance preceding that of the cell body by Nakanishi (1901) and the peculiar division displayed by *B. diphtheriae* in which the split occurs at a point occupied by a metachromatic granule (Williams, 1917).

But with all the observations on the development of bacteria and bacterial cultures, not until recently has anyone found evidence of the existence of complicated life cycles similar to those found among higher Thallophytes or among the Protozoa. Löhnis and Smith (1916) as the result of painstaking researches have, however, reported the finding of such complex cycles in many species of bacteria. They state that all bacteria studied live alternately in an organized and in an amorphous or "symplastic" stage; that bacteria multiply not only by fission, but also by the formation of filterable gonidia, which subsequently germinate either directly or after having entered the symplastic stage. In the symplastic stage they assume the probability of conjugation and describe many complex developmental stages.

The reports of Rosenow (1914) affirming the transmutation of streptococci into pneumococci, and the reverse transformation, and the possibility of transforming a typical diphtheroid organism into a minute coccoid organism and back again (Bunting and Neumann, personal communication) also lend additional

interest to the question of the fixity both of the delicate biologic adjustments of bacteria and of their morphology. That the question is more than an academic one is obvious. Can the bovine tubercle bacillus become metamorphosed into the human type? Can the streptococcus from a streptococcus throat become changed so as to produce a typical scarlet fever, or is the typical scarlet fever merely a different reaction on the part of the individual infected? etc.

It seemed to the authors that some additional information in regard to possible cyclical changes in the development of bacteria might be obtained by making repeated microscopic examinations of organisms at intervals of one to three hours for twenty-four hours after transplanting, and at longer intervals for a week or more.

In all the experiments careful attention was paid to the purity of the original cultures, repeated platings being used in doubtful cases. Typical organisms were transplanted daily to accustom them to the media used and also to eliminate as far as possible the period of "lag" (Barber (1908)). In each series the organisms were grown on tubes from the same lot of media. When in an excellent vegetative state transplants were made. In the first large group of cases a twenty-four hour culture was used as the parent culture, and, in the second group, a forty-eight hour culture. To this parent culture 2 cc. of broth were added and with the resulting suspension four or more tubes were inoculated by seeding with a pipette 0.1 cc. to each tube. The media and broth used were taken directly from the refrigerator and all tubes were placed in the incubator at 37.5°C. at the same time.

In the first group of observations smears were made from the original parent culture and from transplants at the end of 1, 2, 4, 6, 9, 12, 15, 18, 21, 24 and 48 hours and on the seventh day. In each case during the first twelve hours the smear was made from a different and previously untouched culture. The smears made at the fifteen and eighteen hour periods were made from the same tube, care being observed to take the organisms from a different portion of the tube in each case. The rest of the smears were made from cultures that had been used during the

early hours of growth, as it was felt that this disturbance would no longer be an appreciable factor. To insure uniformity of staining, the smears of each strain were for the most part made on a single slide and stained in one operation. Methylene blue was generally used. Observations were made as to size, shape, characteristic grouping, and variation in staining of the bacteria during the development of the cultures.

During these observations, made on a number of strains of many different species of bacteria, it became obvious that the most interesting changes occurred during the first twelve hours of growth. In subsequent observations, therefore, the eighteen and twenty-one hour smears were omitted; thus the necessity of night work was avoided. In addition to the more general observations, careful measurements were made of the organisms from the cultures of different ages by the usual stage and ocular micrometer method. Especial care was taken not to consider two attached organisms as one individual. To avoid error due to individual variations in the organisms, ten organisms selected at random from several different fields were measured. The average of the ten was taken and this average length was used in the construction of curves representing the size of the organisms throughout the growth period. Notation was also made of the smallest and largest organisms observed in the series of ten organisms measured.

Seventy strains were used in all, comprising thirty-seven species. These organisms were obtained either from the American Museum of Natural History, our own collection of stock cultures, or from the other sources mentioned in the appended list.

Coccaceae

1. *Albococcus candicans* no. 260 (American Museum)
2. *Albococcus candicans* no. 174 (American Museum)
3. *Aurococcus mollis* no. 207 (American Museum)
4. *Aurococcus mollis* no. 4 (American Museum)
5. *Staphylococcus aureus* no. 347 (American Museum)
6. *Staphylococcus aureus* (recently isolated stock)
7. *Staphylococcus aureus* (laboratory stock)
8. *Streptococcus M. S.* (Dr. Rosenow)

9. *Streptococcus* no. 736 (Dr. Rosenow)
10. *Streptococcus pyogenes* no. 1 (laboratory stock)
11. *Streptococcus pyogenes* no. 2 (laboratory stock)
12. *Streptococcus pyogenes* no. 3 (laboratory stock)
13. *Streptococcus pyogenes* no. 4 (laboratory stock)
14. *Diplococcus pneumoniae* Type I (Rockefeller Hospital)
15. *Diplococcus pneumoniae* Type II (Rockefeller Hospital)
16. *Diplococcus pneumoniae* Type III (Rockefeller Hospital)
17. *Diplococcus mucosus* no. 92 (American Museum)
18. *Diplococcus pneumoniae* no. 93 (American Museum)
19. *Diplococcus pneumoniae* (from milk)
20. *Diplococcus gonorrheae* no. 288 (American Museum)
21. *Diplococcus catarrhalis* no. 649 (American Museum)
22. A Gram negative coccus (not identified)
23. *Diplococcus catarrhalis* (laboratory stock)
24. *Diplococcus weichselbaumii* (laboratory stock)

Bacteriaceae

25. *B. coli-communis* (laboratory stock)
26. *B. coli-communior* (laboratory stock)
27. *B. typhosus* no. 0 (Wisconsin State Laboratory of Hygiene)
28. *B. typhosus* no. 3 (Johns Hopkins)
29. *B. paratyphosus* A (Wisconsin State Laboratory of Hygiene)
30. *B. paratyphosus* B (Wisconsin State Laboratory of Hygiene)
31. *B. typhosus* no. 1 (laboratory stock)
32. *B. typhosus* no. 2 (laboratory stock)
33. *B. typhosus* no. 4 (laboratory stock)
34. *B. dysenteriae* (laboratory stock)
35. *B. paradysenteriae* (laboratory stock)
36. *B. suipestifer* (laboratory stock)
37. *B. of Friedlander* (laboratory stock)
38. *B. capsulatus* (laboratory stock)
39. *B. proteus* (laboratory stock)
40. *B. prodigiosus* (laboratory stock)
41. *B. pyocyaneus* (laboratory stock)
42. *B. avisepticus* (American Museum)
43. *B. mallei* (American Museum)
44. *B. diphtheriae* no. 1 (laboratory stock)
45. *B. diphtheriae* no. c (laboratory stock)
46. *B. diphtheriae* no. Sp (laboratory stock)
47. *B. diphtheriae* Park no. 8 (American Museum)
48. *B. xerosis*
49. *B. hofmanni* (laboratory stock)
50. *B. hodgkini* (Corbett) (Dr. Bunting)
51. *B. hodgkini* E_3 (Dr. Bunting)
52. *B. hodgkini* (Blessing) (Dr. Bunting)
53. *B. hodgkini* B_3 (Dr. Bunting)

54. *B. hodgkini* M (Dr. Bunting)
55. *B. hodgkini* Endo (Dr. Bunting)
56. *B. hodgkini* Banti II (Dr. Bunting)
57. *B. anthracis* (American Museum)
58. *B. anthracoides* (American Museum)
59. *B. megatherium* (American Museum)
60. *B. mycoides* (American Museum)
61. *B. subtilis* (American Museum)
62. *B. vulgatus* (American Museum)
63. *B. leprae* (American Museum)
64. *B. smegmatis* (American Museum)
65. *Grass bacillus* (American Museum)
66. *B. pertussis* (American Museum)
67. *B. influenzae* (American Museum)

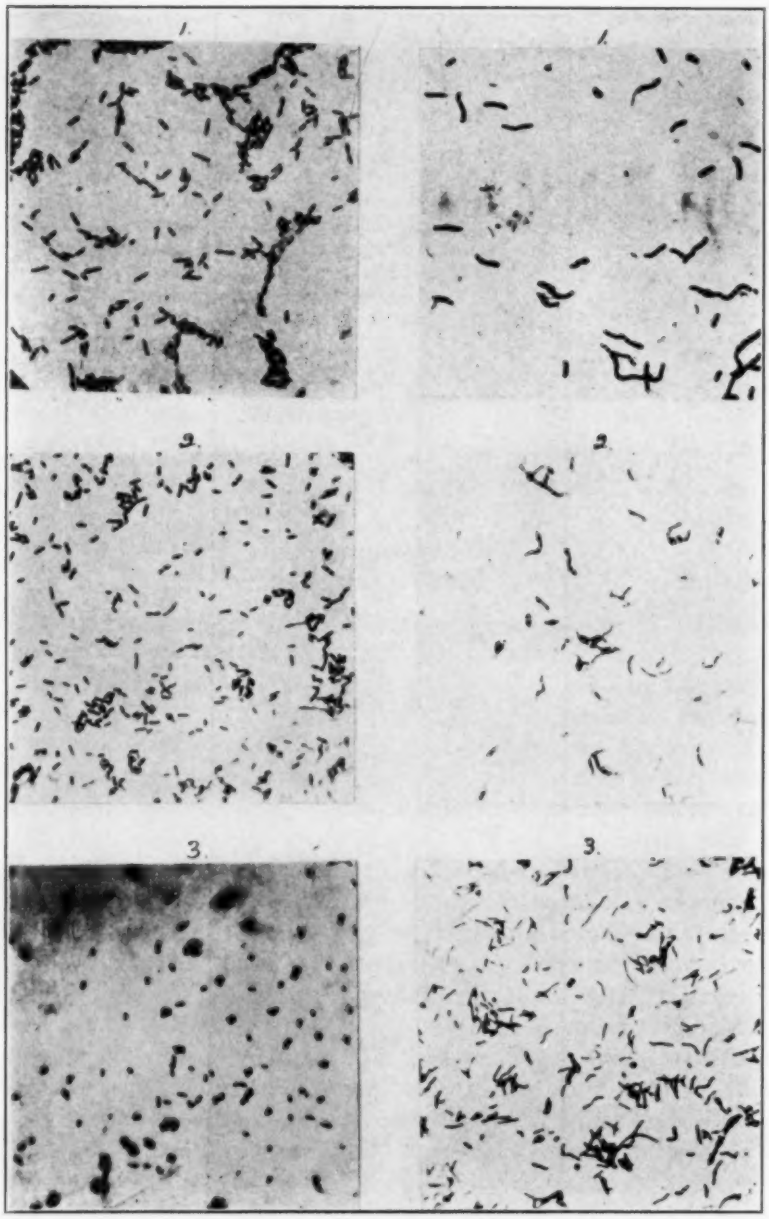
Spirillaceae

68. *Sp. metchnikovi* (American Museum)
69. *Sp. cholerae* (American Museum)
70. *Sp. schuykilliensis* (American Museum)

As a result of these examinations we observed, in all the groups of bacteria studied, striking changes in morphology, especially during the early hours (two to six) of the development of the cultures. In some of the strains no apparent change could be seen for the first two hours, but in the majority of instances even at the end of this initial period a definite increase in size was observed, the organisms took the stain more intensely, and their outline was more sharply defined. Following this, the growth in size was rapid. Many chains of organisms were observed in cultures where they are usually absent. A majority of the organisms from a culture of *Bacillus prodigiosus* four hours old are as large and coarse as the common vegetative forms of the hay bacillus. They will average 1.3 micra in length and 0.7 to 0.8 micra in width. Many of the young, vigorous, rapidly dividing typhoid bacilli from a six hour culture are 4 to 6 micra long and 0.7 to 0.8 micra wide, so thick and clumsy that only further examinations convince an observer that he is dealing with a pure culture of *B. typhosus*. Again, cultures of *Staphylococcus aureus* at this period (four to six hours) present very large, heavy cocci, many of them twice the diameter of the cocci more usually seen. The usual time when the average size

B. DIPHTHERIAE (PARK No. 8). ×800

1. Smear from 4 hour culture
2. Smear from 21 hour culture
3. Smear from 48 hour culture

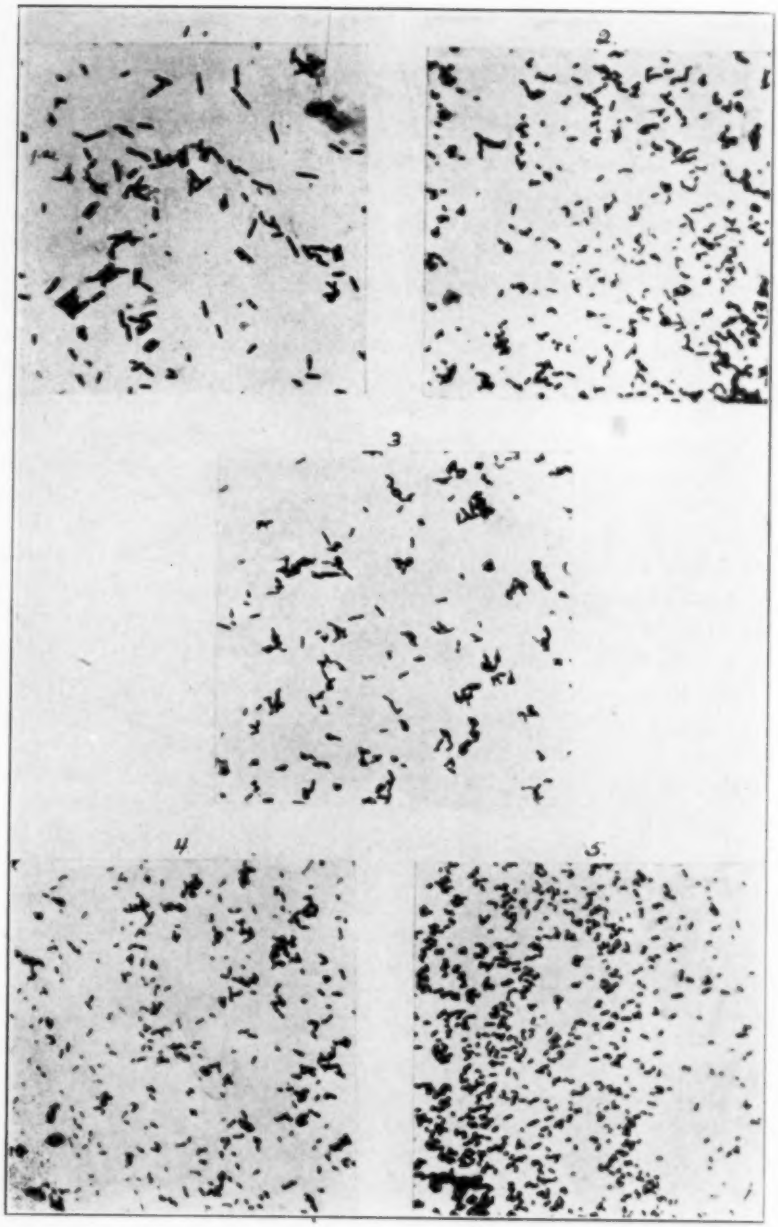


B. PRODIGIOSUS. ×800

1. Smear from 2 hour culture
2. Smear from 4 hour culture
3. Smear from 12 hour culture

SP. CHOLERAE. ×800

1. Smear from 2 hour culture
2. Smear from 12 hour culture
3. Smear from 24 hour culture



B. TYPHOSUS No. 0. ×800

1. Smear from 4 hour culture
2. Smear from 9 hour culture
3. Smear from 15 hour culture
4. Smear from 21 hour culture
5. Smear from 48 hour culture

B. LEPRAE. ×800

1. Smear from 6 hour culture
2. Smear from 12 hour culture
3. Smear from 168 hour culture

B. SUBTILIS. ×800

1. Smear from 6 hour culture
2. Smear from 9 hour culture
3. Smear from 18 hour culture

of the organisms reached its maximum was four to six hours after transplantation. In some instances, however (a strain of *B. coli-communior* for example), the height of the curve was reached in the two hour cultures, and in others (Hopkins strain of *B. typhosus*) not until the cultures had been growing for nine hours. Throughout this period when the organisms are passing, presumably, through the second and third phases according to Buchanan, (1918) the microscopic picture is irregular. The minimum average generation time is short so that the cross section of this rapidly growing culture, which is of course what we obtain when we take a smear, presents organisms in all stages of development, but a majority of them are large and ready for further division.

As the cultures grow older the microscopic picture changes. Smears from the nine or twelve hour cultures present organisms of a smaller average size, this average dwindling, until by the time the cultures are eighteen to twenty-four hours old the classical text-book picture is presented. During this period growth and consequent reproduction are apparently taking place much less rapidly. Chains of bacilli, so prevalent in the younger cultures of the non-chain forming organisms, were not observed. Irregular forms were few in number and a marked uniformity in the size of the organisms was evident. Characteristic grouping manifested itself and the staining was even.

Cultures older than twenty-four and forty-eight hours presented more and more of the involution forms described by many authors. Irregular staining, in many instances bizarre forms, and organisms averaging smaller than those found in the twenty-four hour cultures predominated.

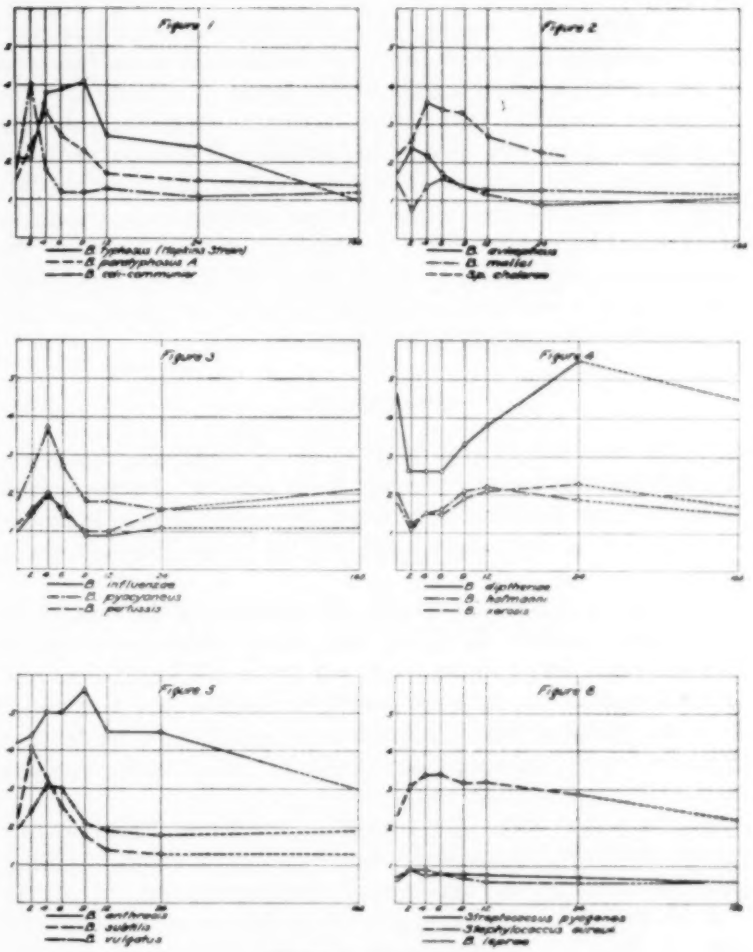
Members of the diphtheria group were the only bacteria (with the possible exception of *B. mallei*) among those examined that did not conform to the general order of morphological changes noted above. In this group, however, the majority of the organisms in the young cultures (two to six hours) were definitely smaller than the organisms transplanted. In fact, in most strains the original average size was not regained until twelve to eighteen hours had elapsed. Not only were the major-

ity of the organisms in the young cultures smaller than those transplanted, but they were also definitely smaller than the involution forms found in cultures six or seven days old. In no other group of organisms did this obtain. In all the others, the old "senile" forms so commonly present in old cultures showed the smallest average size. The peculiar pleomorphism, irregular staining, metachromatic bars and granules so characteristic of the diphtheria group did not ordinarily appear to any great extent until the cultures were fifteen to twenty-one hours old.

The graphs presented (figs. 1–6) are representative of the growth curves obtained and are self-explanatory. The photomicrographs also show clearly the striking differences in the morphology of the different organisms from cultures of different ages.

There would seem to be little need for prolonged discussion of these simple observations. The correlation in time of the occurrence of the long coarse forms with the period when the maximum growth of the culture is taking place, is obvious. That the cross section of the culture with its very short average generation time should show a majority of forms large and nearly ready for further divisions is also obvious. Just why the diphtheria group should depart from the general rule as to the size of the organisms in young cultures is not clear.

The more fundamental question, the reason for the cyclical growth of a bacterial culture or a culture of Paramoecium or other living organisms, is not touched upon by the present observations. As far as the bacterial culture is concerned the conception that the exhaustion of the food supply is an important factor, was overthrown very early. A more adequate conception, generally accepted for many years, is that the bacteria form toxic substances which later inhibit the growth of the organisms themselves. Chesney (1916) has offered strong evidence that lag is also an expression of this injury which the bacterial cell has received in its previous environment. It has long been recognized that many toxic substances which in large concentrations will cause necrosis of the cell will in small concentrations cause proliferation of that cell. Indeed in many,



ORDINATES = Length of Bacteria in Micra
ABSCISSAS = Time in Hours

possibly all, pathological processes which result in a simple hypertrophy or in hyperplasia, it is recognized that injury to the mother cells is the necessary stimulus which initiates this reaction. Jennings has shown that even fertilization in the Infusoria is a stimulus partaking largely of the nature of an injury. Furthermore, Loeb has shown that by mechanical injury to the unfertilized frog's egg he could stimulate cell division and produce an artificial parthenogenetic frog. May it not be possible that the toxic substance which in large amounts cause the death of the bacterial culture and in smaller amounts produce the phenomenon of lag, act when diluted and diffused in a fresh tube of culture medium as the slightly injurious stimulus for the rapid cell division which takes place?

CONCLUSIONS

1. The morphology of seventy strains of organisms, representing thirty-seven species selected from most of the recognized groups of pathogenic bacteria, has been studied at brief intervals for a period of forty-eight hours and at longer intervals for a week.

2. In all instances save among the members of the diphtheria group, and possibly *B. mallei*, the young organisms found in cultures from four to nine hours old are much larger than the forms found in the cultures twenty to twenty-four hours old. In some instances the difference is so great as to render the organisms unrecognizable when viewed by the ordinary standard of the twenty-four hour culture.

3. The period when the largest organisms are found corresponds closely to the period when rapid cell division is taking place as shown by the numbers of viable organisms.

4. In the diphtheria group, however, the young organisms in cultures from four to nine hours old are definitely smaller and more solid staining than the older forms. In fact, the average size in cultures of this age is distinctly less than in the case of very old cultures showing involution forms. In the other groups the senile forms are always the smallest.

5. It is important to recognize the morphological changes in the familiar pathogenic bacteria and to note that these changes occur correlatively with the rapidity of increase in the numbers of organisms.

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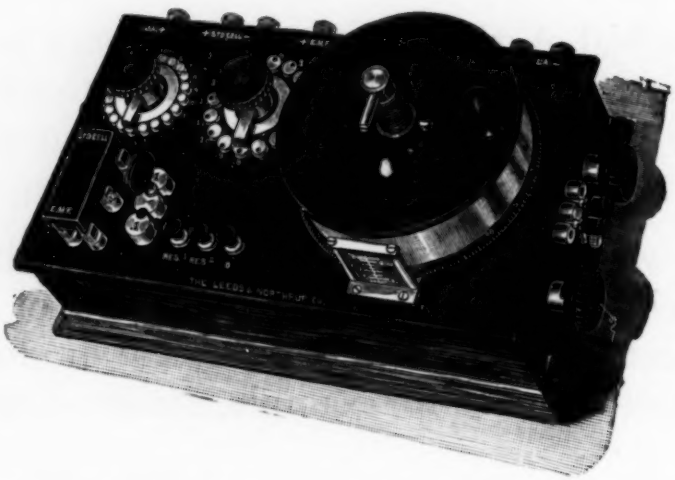

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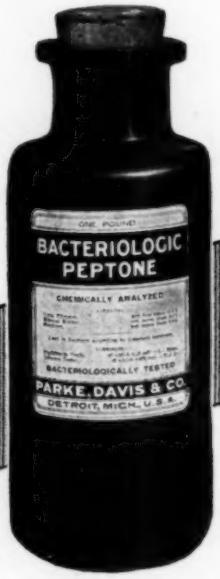

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BACTERIOLOGIC PEPTONE, P. D. & CO., has demonstrated its efficiency in the cultivation of organisms and in the various formulæ in which a standard peptone is necessary. It is used in the biologic analysis of milk, water, foods and soil, in public-health work, and in standardization of disinfectants.

BACTERIOLOGIC PEPTONE, P. D. & CO., is ***uniform in composition, readily and completely soluble, correct in chemical structure.***

Literature and quotations will be promptly supplied on request.

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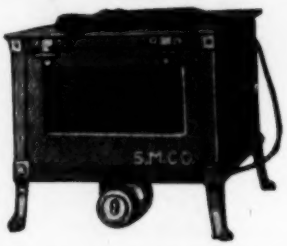
An Inexpensive
High Quality

ELECTRIC DRYING OVEN

Pittsburgh Model

2204. DRYING OVEN (*Sterilizer*), ELECTRIC
PITTSBURGH MODEL



Three heats, approximately 100°, 200° and 300°C. Has no automatic regulator, yet will hold a very even temperature.

Recommended as a sterilizer for glassware and soil bacteria, a drying oven for flour testing and domestic science, and for many other scientific and technical laboratory purposes.

Inside measurement, 10x11x6 inches, with one shelf. Mineral wool between the inner and outer steel walls; nickeled trimmings; glass door; shutter controlled ventilators.

COMPLETE WITH 300°C THERMOMETER, PLUG, AND 6 FEET OF CORD ***$25.00***

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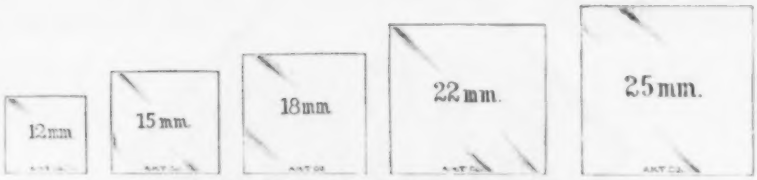

No. 32968 Square Cover Glasses (actual size)

32968. Micro Cover Glasses, Squares, Non-Corrosive, Red Label, of hard crown glass of slightly greenish tint when viewed edgewise, guaranteed against corrosion in any climate. These are the only Cover Glasses we are willing to guarantee against corrosion under all conditions and should not be confused with lower priced glasses made of soft white glass. From the same source and of exactly the same quality as sold by us for the past eighteen years. Neatly packed in round boxes with conspicuous red label. Please specify size and thickness in ordering.

Size, mm.	12	15	18	22	25
No. 1 (from 0.13 to 0.17 mm. thick), **per ½ oz. box**	**1.00**	**.86**	**.86**	**.86**	**.86**
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10% discount in cartons of 50 boxes.
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32969. Micro Cover Glasses, Circles, Non-Corrosive, Red Label, same quality as No. 32968.

Diameter, mm.	12	15	18	22	25
No. 1, **per ½ oz. box**	**1.40**	**1.13**	**1.13**	**1.13**	**1.13**
No. 2, **per ½ oz. box**	**1.13**	**.86**	**.86**	**.86**	**.86**

10% discount in cartons of 50 boxes.
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32995. Micro Slides, Non-Corrosive, Red Label, of hard glass of slightly greenish tint when viewed edgewise; guaranteed against corrosion in any climate. With well ground edges and of fair uniformity in thickness, i.e., about 1 mm. We recommend this as the best Micro Slide now available.

Size, inches.	3 x 1	3 x 2
Per ½ gross carton	**.60**	**.75**

10% discount in case containing 50 cartons.

32977. Micro Slides, White Glass, of uniform thickness, with edges well ground. The manufacturer of the glass of which these slides are made claims it to be non-corrosive under all conditions and in any climate. As the manufacture is under scientific control, we believe this statement to be true, but as practical confirmation of this claim requires an experience of several years in both distribution and use of the slides, we feel that such confirmation is as yet impossible in view of the short time these slides have been available. Size 3 x 1 inches.

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Have Opened up to the Chemist and Bacteriologist Entirely New Fields of Investigation

We are Prepared to Furnish All of the Various Types of Equipment Required for Such Investigation

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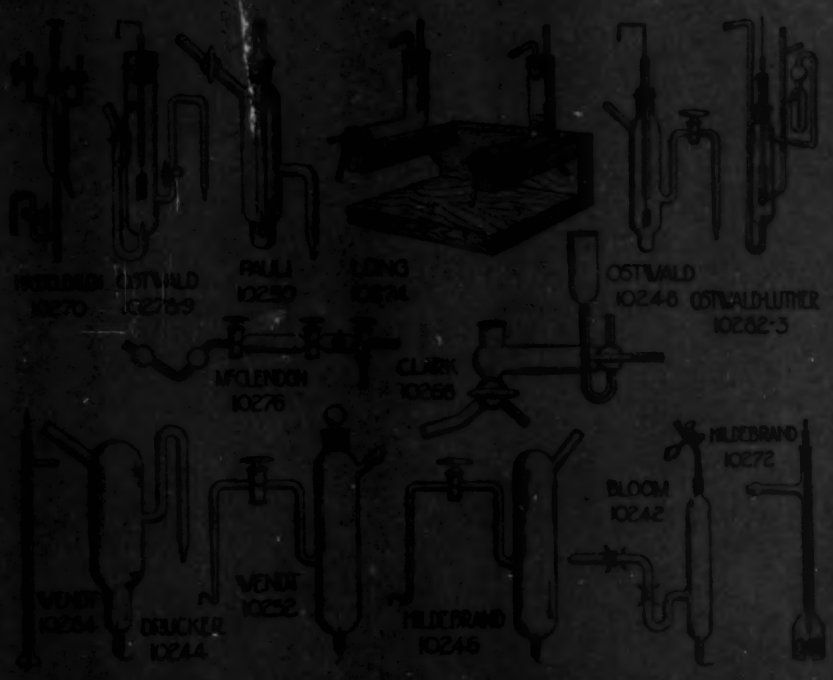
Now Being Constructed in Our Glass Shop



In addition to the glass cells shown above, we manufacture all of the measuring instruments required. We shall be pleased to make suggestions or give information as to the apparatus required and the methods to be employed for such investigations.

CENTRAL SCIENTIFIC COMPANY

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BACTO-PEPTONE

DIFCO STANDARDIZED

The Original Standardized Peptone for Bacteriology

Bacto-Peptone is the original peptone designed to meet particularly the nutritional requirements of bacteria in culture.

It is the first peptone designed to meet the requirement of uniformity in its chemical and biological properties.

It is made in large quantities by expert workmen under rigid scientific check and control. Raw materials are approved only after assay.

Accurate records of each lot are kept throughout the manufacturing process, and only those lots which pass definite chemical and bacteriological tests are allowed to go into stock for distribution.

A definite requirement as to the H-ion concentration in solution was applied first to Bacto-Peptone.

It is standardized with the Tryptophane content and supports vigorous indol production within 24 hours after inoculation.

Bacto-Peptone has passed the experimental stage and has proved its absolute dependability for all nutritional requirements of Bacteriology.

Bacto-Peptone is made to our matrix and completely standardized in all batches made for sale.

The additional equipment which we were forced to install in order to furnish the unprecedented quantities of Bacto-Peptone required for the U. S. and allied military establishments enables us to take care of all orders, irrespective of size, promptly and efficiently.

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